

THE EFFECTS OF AN ELECTRIC FIELD ON SOLUBLE COLLAGEN

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SUMMARY

Electric birefringence decay curves of collagen suspended in aqueous buffered media were plotted as functions of pulse width and amplitude. They were then resolved into two components by means of an analog simulator. When these data were combined with the results of repeated pulsing, it was shown that an electric field promotes aggregation of collagen, although the variety of aggregate sizes falls within a fixed range. Observations of electric birefringence of dissolved collagen preparations as a function of ionic strengths tend to indicate that the bonding that occurs in an electric field is electrostatic.

INTRODUCTION

It has been observed that the presence of an electric field induces changes in the permanent dipole moment and electric susceptibility of dissolved collagen [1]. This is not unexpected because of the large distributed electric charge pattern of the collagen molecule and its many polar groups. To examine more closely the effects of an electric field on collagen, a study was carried out in which electric birefringence in a single pulsed field was measured as functions of pulse width and pulse amplitude, and samples were subjected to trains of pulses to investigate the effect of repeated application of an electric field.

Particular attention was paid to the electric birefringence decay curve because of its relation to the rotatory diffusion coefficient. When the logarithm of electric birefringence is plotted versus decay time for a polydisperse suspension, a curved line results which can, in principle, be resolved into components corresponding to each species of aggregate present. Because of the nature of exponential decay curves, however, it is very difficult to resolve a composite curve of more than two species [2]. Regardless of the number of components that contribute to such a curve, a mathematically valid resolution into two apparent components is almost always possible. To alleviate this difficulty in this work, electric birefringence decay curves were resolved into two components: One was arbitrarily assigned the rotatory diffusion coefficient of collagen monomer [3], 1050 s^{-1} , since it could be assumed that some

monomer was present in all of the collagen preparations; and the other rotatory diffusion coefficient, as observed, was considered an average for all aggregated collagen species present, and thus served as a measure of the extent of aggregation.

EXPERIMENTAL

The source of the soluble collagen used in this work was fresh calfskin corium. This was placed in contact with citrate buffer at pH 3.40 and ionic strength 0.40 in an automated apparatus which maintained constant electrolytic conditions during the dissolving process. This technique has been described in detail elsewhere [4]. The resulting collagen suspensions were subsequently dialyzed to other solution conditions and were verified by electron microscopic examination of reconstituted fibers and by sedimentation pattern and showed a coefficient of 3.0 S. Collagen concentration was determined via Kjeldahl nitrogen on the basis that collagen contains 17.5% nitrogen. All samples were held at 10 °C, except when electric birefringence measurements were being carried out, which was done at 24–26 °C. Samples remained at the latter temperature for 1–4 h. The design of the electric birefringence apparatus has been given in previous publications [1, 3]. The temperature rise in the sample due to the pulsed field was calculated on the basis of an 8-ms pulse of 3300 V/cm field strength and the assumption that all heat in the sample cell was generated between the electrodes, which represents the severest conditions that could be encountered in this research program. Under these conditions the temperature rise was less than 0.5 °C.

Resolution of the decay curves into two components was accomplished through the analog simulator shown in Fig. 1. This consists of two channels which simultaneously generate two exponential decay curves. One channel yields a decay curve corresponding to collagen monomer, and the other gives a curve corresponding to an average for the collagen aggregates present. The potentiometers n_{0A} and n_{0B} set the amplitude of the curve at zero decay time for each of the two components, and

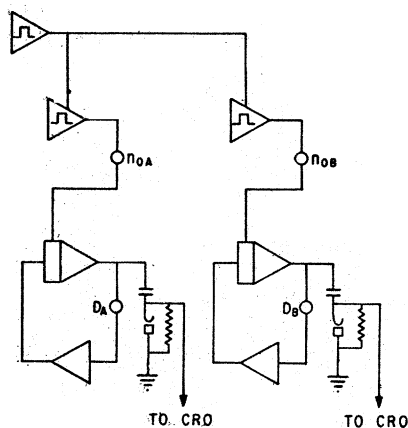


Fig. 1. Analog simulator for resolving electric birefringence decay curves. Potentiometer D_A is set to the rotatory diffusion coefficient of collagen monomer; D_B determines the average rotatory diffusion coefficient of collagen aggregates; n_{0A} and n_{0B} determine the contribution to electric birefringence of monomer and aggregates, respectively, at pulse termination.

potentiometers D_A and D_B set the values of the rotatory diffusion coefficients. The oscilloscope can display the curve generated by either channel or the sum of both.

In use, the composite electric birefringence decay curve was plotted on the oscilloscope graticule with a grease pencil as angular birefringence versus decay time. Potentiometer D_A was set to the value of the rotatory diffusion coefficient of collagen monomer. The other three potentiometers were then adjusted until the displayed sum trace matched the curve plotted on the graticule. Reading the calibrated dials of the potentiometers or displaying each channel separately yielded values of D_B , the average rotatory diffusion coefficient for all aggregated species, and the value of the electric birefringence at the termination of the applied pulse (zero decay time) for both monomer and aggregates.

RESULTS AND DISCUSSION

A graph of D_B , the average rotatory diffusion coefficient for aggregated collagen at constant pulse amplitude versus pulse width, is shown in Fig. 2. For the data shown, the collagen concentration was approx. 0.30% and the ionic strength 0.05, but the shape of the curve appears to be typical of all soluble collagen preparations. It is observed that there are plateau values for D_B at 750 and 90 s^{-1} , which

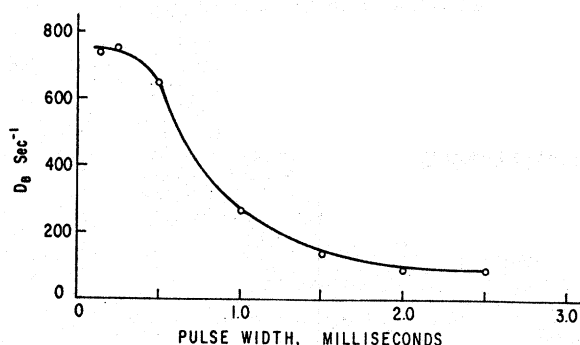


Fig. 2. Graph of average rotatory diffusion coefficient of suspended collagen aggregates (D_B) versus pulse width in electric birefringence. Collagen concentration approx. 0.30%; electric field strength is 3300 V/cm and electric birefringence was carried to the steady saturated state.

means that the range of aggregates stays within definite limits. The latter value of rotatory diffusion coefficient corresponds to a particle having a length equal to twice that of collagen monomer. This does not necessarily mean that the largest particle present is an end-to-end dimer because a staggered side-to-side structure of several particles having an overall length equal to that of dimer is possible, and it would be difficult to detect this through the use of equations which relate rotatory diffusion coefficient to particle dimensions. The limiting value of 750 s^{-1} at very small pulse widths is evidence that some collagen aggregates were present in the preparation even before the electric field was applied, since it is smaller than the rotatory diffusion coefficient of monomer.

The progressive decrease in D_B with increasing pulse width indicates that either the electric field is inducing aggregation, or that there is an electrostatic interaction

between neighboring suspended collagen particles. To decide this, decay data were plotted in semilogarithmic fashion for several values of ionic strength as shown in Fig. 3.

The increase in the slope of the lines with increasing ionic strength until the latter reaches a value of about 0.0070 indicates a corresponding increase in rotatory diffusion coefficient. Increasing ionic strength would enhance the ion atmosphere of the collagen sample building up a form of electrostatic shielding between suspended

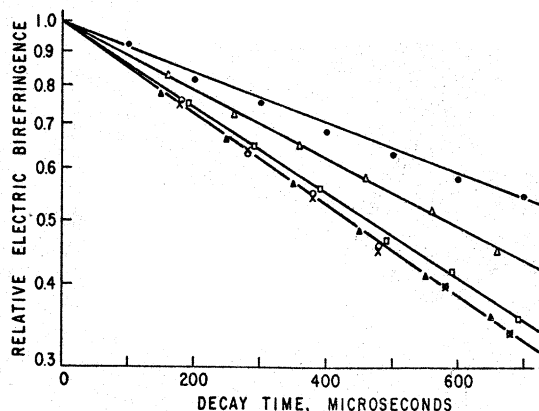


Fig. 3. Graph of electric birefringence decay curves of dissolved collagen at several values of ionic strength. Collagen concentration, approx. 0.30%; electric field strength, 1250 V/cm; pulse width, 8 ms; ionic strength; ●, 0.0020; △, 0.0040; □, 0.0050; ○, 0.0070; ▲, 0.010; ×, 0.016.

collagen particles, and this would diminish the effect of coulombic forces. Since the decay curves observed at values of ionic strength higher than 0.0070 fall on the same line, it can be concluded that particle interaction takes place only at lower values of ionic strength. The plotted lines in this figure appear straight because of the short time interval covered. Had the time base been compressed so as to show decay time beyond 700 μ s, curvature would become apparent.

The relation between the electric birefringence due to monomeric collagen and that due to collagen aggregated in an electric field is shown in Fig. 4. At low values of pulse width monomeric collagen makes a larger contribution to the electric birefringence, but after (in the case of this particular sample) about 1.27 ms, aggregation has progressed to the point where the aggregated species account for a greater portion. This is, of course, a natural consequence of a population of aggregates being built at the expense of monomeric units.

The effect of repeatedly pulsing a dissolved collagen preparation is shown in the electric birefringence pattern of Fig. 5, where the upper trace follows the applied electric field and the lower trace is the optical response of the system. Here it is seen that electric birefringence increases with each succeeding pulse until a maximum is reached. If a time interval of the order of 20 s was allowed to elapse after a train of five or six pulses had been applied, the sample reverted to its original condition, and on applying a second train of pulses, the electric birefringence dropped to its original value and increased to a maximum as in the case of the original observation.

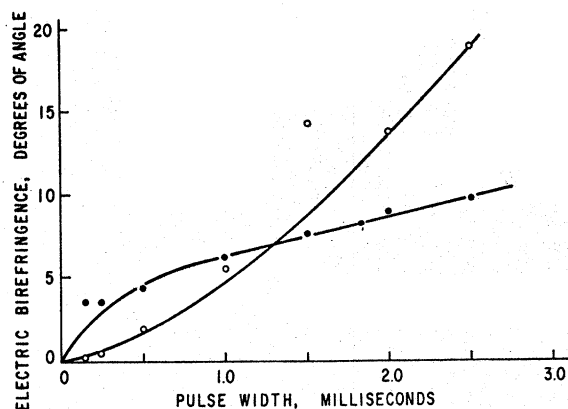


Fig. 4. Graph of contributions of monomeric and aggregated collagen to electric birefringence versus pulse duration. Collagen concentration, 0.69%; electric field strength, 3300 V/cm; ○, aggregated collagen; ●, monomeric collagen.

Further repeated pulsing eventually caused a transparent film of collagen to form on the cathode of the electric birefringence cell. This pattern was obtained at a collagen concentration of 0.56%, but appears to be typical of all soluble collagen preparations.

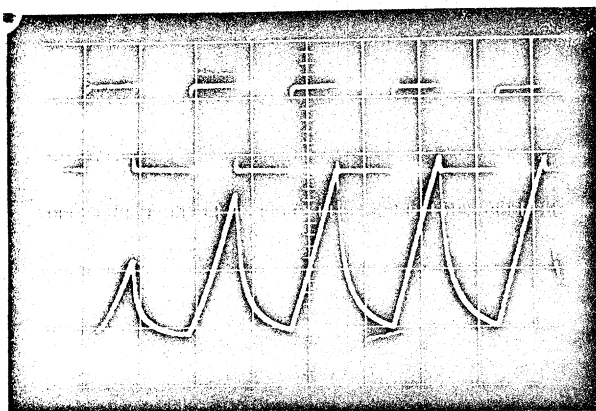


Fig. 5. Electric birefringence pattern showing effect of repeatedly pulsing a dissolved collagen preparation. The upper trace is the applied field, and the lower trace is the optical response of the system. The abscissae are $2 \mu\text{s/cm}$.

These observations lead to the conclusion that the presence of an electric field promotes aggregation of collagen particles suspended in an aqueous buffered medium. In fields of short duration, the aggregation is of a transient nature, but the bonding becomes progressively stronger as field strength and duration increase, and possibly eventually becomes permanent. The bonds formed are probably of an electrostatic nature since bond formation is promoted by reducing ionic strength, as shown in Fig. 3.

REFERENCES

- 1 Kahn, L. D. and Witnauer, L. P. (1971) *Biochim. Biophys. Acta* 243, 388
- 2 Gardner, D. G., Gardner, J. C., Lausch, G. and Meinke, W. W. (1959) *J. Chem. Phys.* 31, 978
- 3 Kahn, L. D. and Witnauer, L. P. (1969) *J. Am. Leather Chem. Assoc.* 64, 12
- 4 Kahn, L. D. and Witnauer, L. P. (1969) *J. Appl. Polym. Sci.* 13, 141